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Xylem dysfunction caused by water stress and freezing in two species of co-occurring chaparral shrubs

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ABSTRACT

Water transport from the roots to leaves in chaparral shrubs of California occurs through xylem vessels and tracheids. The formation of gas bubbles in xylem can block water transport (gas embolism), leading to shoot dieback. Two environmental factors that cause gas embolism formation in xylem conduits are drought and freezing air temperatures. We compared the differential vulnerabilities of *Rhus laurina* and *Ceanothus megacarpus*, co-dominant shrub species in the coastal regions of the Santa Monica Mountains of southern California, to both water stress-induced and freezing-induced embolism of their xylem. *Rhus laurina* has relatively large xylem vessel diameters, a deep root system, and a large basal burl from which it vigorously resprouts after wildfire or freezing injury. In contrast, *Ceanothus megacarpus* has small-diameter vessels, a shallow root system, no basal burl and is a non-sprouter after shoot removal by wildfire. We found that *R. laurina* became 50% embolized at a water stress of –3 MPa and 100% embolized by a freeze–thaw cycle at all hydration levels. In contrast, *C. megacarpus* became 50% embolized at a water stress of –9 MPa and 100% embolized by freeze–thaw events only at water potentials lower than –3 MPa. Reducing thaw rates from 0·8 °C min^{-1} to 0·08 °C min^{-1} (the normal thaw rate measured *in situ*) had no effect on embolism formation in *R. laurina* but significantly reduced embolism occurrence in well-hydrated *C. megacarpus* (embolism reduced from 74 to 35%). These results were consistent with the theory of gas bubble formation and dissolution in xylem sap. They also agree with field observations of differential shoot dieback in these two species after a natural freeze–thaw event in the Santa Monica Mountains.

Key-words: chaparral; embolism; freezing; water stress; xylem.

INTRODUCTION

Although drought- and freezing-induced xylem embolism (gas blockage) has been the subject of several recent studies (Cochard & Tyree 1990; Sperry & Sullivan 1992; Sperry *et al.* 1994), the ecological and evolutionary significance of such embolism remains poorly understood. The mechanism of gas embolism formation in the woody stems of vascular plants is not fully resolved, but it appears to depend upon the type of environmental stress causing gas bubble formation in xylem sap (Fig. 1a). Two of the most common environmental stresses are summer drought (Kolb & Davis 1994; Redtfeldt & Davis 1996) and winter freezing (Wang, Ives & Lechowicz 1992; Sperry 1995). Available evidence suggests that water stress-induced embolism is not a direct function of the size of xylem conduits, but of the maximum pore size in the cell walls of vessels and tracheids (Sperry & Tyree 1988; Sperry & Tyree 1990; Cochard, Cruiziat & Tyree 1992; Jarbeau, Ewers & Davis 1995). In contrast, freezing-induced embolism is strongly correlated with the diameter or volume of xylem conduits (Ewers 1985; Cochard, Cruiziat & Tyree 1992; Sperry & Sullivan 1992; LoGullo & Salleo 1993). Evidently, conduit size determines the size of the gas bubbles forced out of solution during a freezing event. Large gas bubbles formed in large conduits are theoretically more prone to expansion and more resistant to dissolution at the time of thaw than are small gas bubbles in small conduits (Yang & Tyree 1992; Sperry 1995).

Because water expands when it turns to ice, there may be a measurable increase in the xylem pressure of woody stems as they freeze. This pressure increase may later facilitate dissolution of bubbles at the time of thaw (Hammel 1967; Robson, McHardy & Petty 1988). In contrast, a decrease in xylem pressure due to water stress or the onset of transpiration during a thaw event may hinder dissolution. Consistent with this notion, Tyree & Yang (1992) controlled xylem pressures during thaw events in the woody stems of sugar maple, *Acer saccharum*, and found that dissolution of bubbles into xylem sap halts at pressures more negative than –6 kPa. Also, Sperry *et al.* (1994) have recently shown that lower water potentials generally increase vulnerability to freeze-induced embolism.

The degree of xylem embolism caused by a freeze–thaw event may also depend on the rate of thaw, although data are limited (Ewers 1985; Tyree & Yang 1992). In theory, fast thaws would give bubbles less time to dissolve and cause cavitation at less negative pressures (Sperry 1995).

The coastal chaparral community of southern California provides a particularly valuable system to investigate the interactive effects of water stress and freezing on xylem embolism. Mature chaparral vegetation of southern

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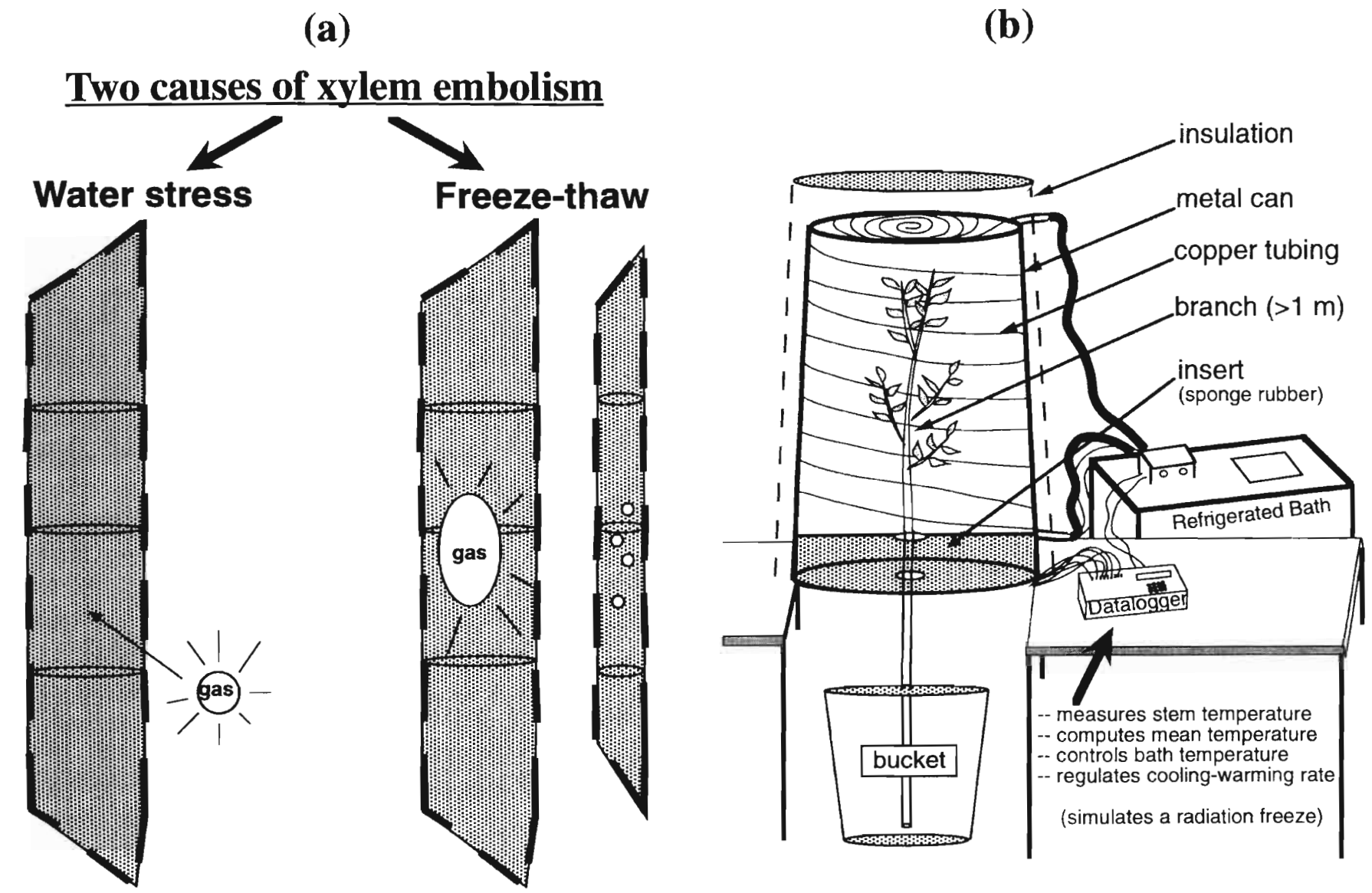

Figure 1. (a) Diagram comparing two different causes of xylem embolism formation: water stress and freeze–thaw events. Water stress-induced embolism in chaparral shrubs occurs as a function of the size of the pores in the pit membrane of cell walls as fully described by Jarbeau, Ewers & Davis (1995). Freezing-induced embolism is a function of the diameter of the xylem vessel which evidently determines the maximum diameter of internal gas bubbles as suggested by Sperry & Sullivan (1992). (b) Diagram of the portable chilling apparatus used to simulate a natural freeze–thaw event of branch tissue. The insulated aluminium can cools the branch by the flow of chilled methanol through copper tubing along the inside surface of the can. All internal surfaces are painted black to facilitate radiative heat exchange. The rates of cooling and thawing are controlled by a computer program downloaded to a datalogger, which controls the temperature of the methanol in the water bath. Measurements from thermocouples positioned on the branch are recorded by the datalogger.

California is composed of evergreen, sclerophyllous shrubs which remain physiologically active throughout the year (Mooney, Harrison & Morrow 1975; Davis & Mooney 1986). This suggests that the water transport system of such species must remain functional during the events of summer drought and winter frosts. Near the coastline, the oceanic influence ameliorates temperature fluctuations so that nighttime air temperatures in winter rarely reach 0 °C. In contrast, just a few kilometres inland, mountain valleys frequently collect cold air during winter nights causing air temperatures to drop as low as –12 °C (Boorse 1995). During unusually dry years, the normally 5–6 month summer drought season can extend into December (Kolb & Davis 1994), when coldest seasonal air temperatures are reached. Under such conditions, seasonal drought in combination with nighttime frost could potentially enhance the occurrence and severity of xylem embolism formation.

One of our study sites is dominated by two species of chaparral shrubs known to have contrasting life history characteristics and divergent responses to summer drought and freezing air temperatures (Thomas & Davis 1989; Saruwatari & Davis 1989; Misquez 1990). *Rhus laurina* Nutt. (laurel sumac) has a large root crown facilitating nearly 100% resprout success after shoot death by wildfire (Zedler, Gautier & McMaster 1983; Thomas & Davis 1989) or freezing events (Mooney 1977; Misquez 1990). This species has deep roots and the largest vessel diameters reported for chaparral plants (Carlquist & Hoekman 1985), and maintains a high seasonal water potential (DeSouza, Silka & Davis 1986; Frazer & Davis 1988). In contrast, *Ceanothus megacarpus* Nutt. (bigpod ceanothus) is devoid

of a root crown, does not resprout after shoot removal by fire or other disturbance (non-sprouter), has shallow roots (Thomas & Davis 1989), has relatively narrow vessels (Carlquist & Hoekman 1985), and undergoes severe tissue dehydration during each summer drought period (Gill & Mahall 1986; Kolb & Davis 1994).

In southern California, both *R. laurina* and *C. megacarpus* are restricted to fairly low elevation, coastal sites (Schlesinger *et al.* 1982; Misquez 1990). However, *R. laurina* may occur, if anything, at slightly colder sites than *C. megacarpus*. For instance, according to Hickman (1993), in southern California *R. laurina* occurs at elevations up to 1000 m, whereas C. *megacarpus* is restricted to coastal sites up to 750 m. In exceptional cases *C. megacarpus* may occur at elevations up to 1200 m, but these are at ridge crests of south-facing, coastal exposures (Schlesinger *et al.* 1982), locations that would tend to moderate the minimum winter temperatures. At least at our 'cold' study site, which is located 4 km inland from our 'warm' study site in the Santa Monica Mountains, *R. laurina*, although subject to periodic dieback due to winter freezes, occurs at colder microsites than does *C. megacarpus* (S. Davis, unpublished results). Near the inland, winter-cold margins of their distribution in the Santa Monica Mountains, we observed *R. laurina* to undergo total shoot dieback when exposed to a winter freezing event (26 December 1990), whereas adjacent *C. megacarpus* remained uninjured (Fig. 2c). It is possible that low temperatures limit the distribution of both of these species, with *R. laurina* occurring at colder sites than *C. megacarpus* based solely on its ability to resprout following freezing damage (Misquez 1990).

Based upon life-history patterns (*R. laurina* can resprout from the root crown following fire or other disturbance whereas *C. megacarpus* is a non-sprouter), vessel diameters (*R. laurina* has vessels that are about twice as wide as those of *C. megacarpus*) and seasonal measurements of water potentials (*C. megacarpus* exhibits much lower water potentials during summer drought), our hypothesis was that stems of *R. laurina* would be more vulnerable to both drought-induced and freezing-induced embolism than those of *C. megacarpus*. Our objectives were (1) to monitor natural winter freezing events at the winter-cold, inland edge of the range of *R. laurina*, (2) to determine the interactive effects of water stress and freeze–thaw events on xylem dysfunction in two representative species of chaparral shrubs (*R. laurina* and *C. megacarpus*), (3) to determine the influence of thaw rate on xylem embolism formation, and (4) to explore the possibility that freezing-induced embolism in combination with drought could limit the survival and distribution of these species in nature.

MATERIALS AND METHODS

Study sites

The 'warm' study site is located in a natural stand of mixed chaparral, near Pepperdine University's Biological Preserve in Malibu, at an elevation of 280 m, in a coastal exposure of the Santa Monica Mountains (34°2'30"N, 118°42'30"W). Plant community structure and pre-fire as well as post-fire seasonal dynamics at this site have been described elsewhere (Saruwatari & Davis 1989; Thomas & Davis 1989). The plants sampled were between 22 and 23 years old, having previously burned in the Malibu wildfire of 1970 (Los Angeles County Fire Department).

The 'cold' study site is located 4 km inland from Malibu at Cold Creek Canyon, where nighttime temperatures during winter months can be 12 °C lower than at Malibu (Boorse 1995). The cold site was used to monitor air, stem and leaf temperatures of chaparral shrubs *in situ* during natural freeze–thaw events and thereby determine normal rates of cooling and warming under field conditions. These rates were then simulated in the laboratory to provide realistic freeze–thaw treatments of large chaparral branches (see below). In addition, leaf viability measurements following the methods of Boorse (1995) were made before and after a natural freeze–thaw event at the cold site, and xylem embolism measurements of native plants were made at both study sites, as described below.

Monitoring temperatures at Cold Creek Canyon

We monitored air, leaf and stem temperatures at Cold Creek Canyon using two dataloggers (Model 21X, Campbell Scientific, Inc., Logan, UT), placed 150 m apart. One datalogger was located near a ridge crest (upper datalogger) at the lowest elevational limits of *R. laurina* distribution at our study site (presumably limited by the much colder valley temperatures that occur on calm, clear nights; see dashed line in Fig. 2A). The other datalogger (lower datalogger) was placed on the valley floor (bottom arrow in Fig. 2A). Six 36 gauge thermocouples were used to measure air temperature, leaf temperature, and stem temperature at the top of the canopy (≈2 m) and in the understory (≈0·5 m) (cf. Fig. 2B). *R. laurina* was the species used at the upper datalogger. Since *R. laurina* did not occur on the valley floor, *R. ovata* was substituted and monitored by the lower datalogger (Fig. 2A, bottom arrow). *R. ovata* was chosen as a surrogate for *R. laurina* because of its close taxonomic affinity (Young 1974) and similarity in leaf size, leaf shape, canopy structure and seasonal water status (Poole & Miller 1975; Miller & Poole 1979).

Leaf thermocouples were attached to leaf petioles with masking tape and spring-loaded so that their thermal junction was in firm contact with the mid-region, abaxial surface of the blade. Stem thermocouples were inserted in a small hole drilled into the bark of ≈6-mm-diameter stems and taped into position so that the thermal junction of the thermocouple was adjacent to the outermost xylem. Temperatures were measured once each minute, averaged for each 10 min interval throughout the day and stored during what is normally the coldest period of the year, December 1992 to January 1993 (cf. Boorse 1995). Between 0300 and 0400 h on cold, clear nights, leaf temperatures of topmost leaves were found to be on average 0·3–0·4 °C lower than air temperatures and 0·8–1·4 °C

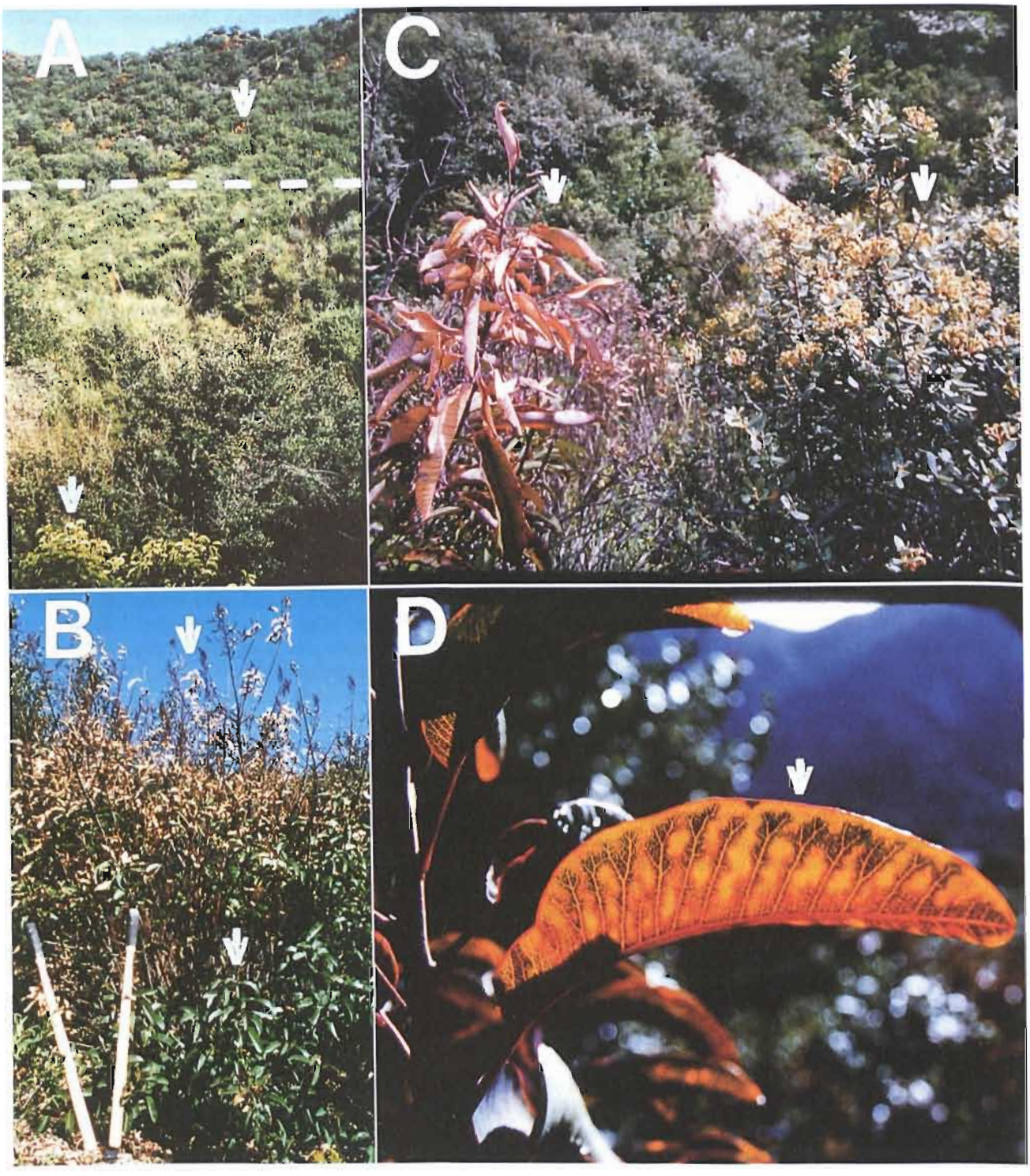

Figure 2. Photographs taken at our Cold Creek Canyon study site in the Santa Monica Mountains in late January 1993, about 1 month after the nighttime freezing events of 19 and 20 December 1992 (see Fig. 3). (A) All individuals of *Rhus laurina* are restricted to warmer hill tops (denoted by the white line) and show evidence of freezing injury to their upper canopies (top arrow). In contrast, *Rhus ovata* is unrestricted in its distribution from the hill top to the cold valley floor, about 200 m below the ridge crest (bottom arrow) and had no visual evidence of freezing injury. *Ceanothus megacarpus* does not grow at this inland site but is replaced by C. *crassifolius*. (B) Close-up view of *R. laurina* showing evidence of severe injury to topmost leaves and stems (top arrow) but lack of injury to understory leaves and stems (bottom arrow) (cf. Fig 3). (C) Comparison of *R. laurina* (left arrow) and *C. megacarpus* (right arrow) growing adjacent to each other at a microsite about 1 km closer to the ocean and at a higher elevation (warmer) than shown in Figs 2A and 2B. *R. laurina* shows evidence of freezing injury to upper canopy leaves whereas *C. megacarpus* does not. (D) Close-up of a *R. laurina* leaf 3 d after the freezing injury of 19–20 December 1992. The mottled appearance prevented us from assessing freezing injury by a colour index using a Munsell Color Chart (Boorse 1995). We thus relied on measurements of variable fluorescence (F_v/F_m) and estimation of the percentage of leaf mesophyll cells alive using the vital stain fluorescein diacetate (Table 1).

lower than bottom-most leaves (one-way ANOVA, $P < 0{\cdot}001$ to $P < 0{\cdot}01$). However, temperatures of topmost leaves and topmost stems were not significantly different (range in $P > 0{\cdot}3$ to $P > 0{\cdot}9$; the mean difference in temperatures of topmost leaves and stems for six of the coldest nights varied between 0·01 and 0·07 °C). Since cell viability tests and visual assessment of injury were performed on leaf tissues, we restricted our analysis to leaf temperatures instead of stem temperatures during episodes of night time freezes.

Measuring leaf viability at Cold Creek Canyon

Chlorophyll fluorescence

Freezing injury to the upper canopy (top leaves) and understory (bottom leaves) of *Rhus laurina* and *R. ovata* were assessed using photosynthetic fluorescence (ratio of variable to maximum fluorescence; F_v/F_m; Bolhar-Nordenkampf *et al.* 1989). F_v/F_m was determined before and after the freezing events of 19 and 20 December 1992. Measurements were made near midday on dark-adapted leaves (dark cuvettes on leaves for 30 min prior to readings) on 16, 19, 20, 21, 23 and 30 December, using a chlorophyll fluorescence meter (Model CF-1000, P. K. Morgan Instrument Inc., Andover, MA) set at an exposure time of 15 s and an actinic light level of 500 μmol m^{-2} s^{-1} PFD. Post-freeze measurements of F_v/F_m (after 20 December) were performed to detect the possibility of fluorescence recovery. Two leaves from the upper canopy and two leaves from the understory of five individuals of each species ($n = 5$) were permanently tagged and used for all measurements.

Vital stain

One week after the last fluorescence measurement on 30 December 1992, leaves which were previously tagged for F_v/F_m measurements were harvested at dawn, immediately placed in sealed plastic bags on ice, and returned to the laboratory within 30 min to undergo vital staining following the methods of Boorse (1995). Five cross-sections ≈100 mm thick were cut from the blade of each leaf in deionized water using a new razor blade. The sections were immediately placed in 10 cm^3 of deionized water, after which 0·1 cm^3 of a freshly prepared 0·01% solution of fluorescein diacetate in acetone was added (Widholm 1972). After 1 min, an additional 10 cm^3 of water was added to the five sections. The thinnest section from each leaf was mounted onto a microscope slide and viewed under blue fluorescent light (Excitation filter BG-12) in an epi-fluorescent microscope (Microphot-FX, Nikon Inc., Garden City, NY) under 100X magnification. Starting at the midrib of each blade, which served as a standard reference for all leaves, and to avoid damage to edges, since that is where forceps were used, 100 palisade parenchyma cells were counted and scored as to whether they fluoresced yellow-green (alive) or exhibited red, Chl *a* autofluorescence (dead). The number of cells alive was divided by the total number of cells counted and the decimal fraction was multiplied by 100 to obtain the percentage of cells alive.

Native embolism of stem xylem

Also during the early part of January, one branch from each of 12 individuals of *Rhus laurina* and 12 individuals of *R. ovata* from the cold site at Cold Creek Canyon and from the warm site at Malibu were harvested and used to estimate xylem embolism by the method described below.

Measurement of xylem embolism

We used the Sperry method (Sperry, Donnelly & Tyree 1988) to estimate the percentage loss in hydraulic conductivity due to gas emboli (% loss in K_h) in stems of *R. laurina* and *C. megacarpus*. The degree of embolism measured was either a result of native levels that naturally occur in the field (control group) or native levels plus additional embolism caused by artificial treatments of water stress or freeze–thaw events (treated group). In order to avoid the artificial introduction of gas bubbles into xylem conduits at the time of sampling, maximum vessel lengths for each species were determined by the air injection method of Zimmermann & Jeje (1981). Mean maximum vessel length was 1·1 ± 0·09 m (SE, $n = 10$) for *R. laurina* and 0·8 ± 0·07 m for *C. megacarpus*. Only branches about twice as long as the maximum vessel length were cut from mature individuals of each species in the field. All samples were cut soon after sunrise (0600 to 0800 h) and placed in a moist plastic bag to avoid dehydration during the sampling procedure. Branches were returned to the laboratory within 30 min after they were cut.

In the case of 'hydrated treatments', the cut ends of branches were immediately placed in a bucket of water in the field, twice trimmed at 20 cm increments under water, shaved with a new razor blade and left underwater, with the leafy branches left above water but covered with a moist plastic bag.

In the case of 'dehydrated treatments', upon arrival in an air-conditioned laboratory, the bag was removed to allow bench-top drying of the branches for one to several days, at which time the bag was replaced overnight to allow equilibration of xylem pressure throughout the branch system before determination of water potential by the pressure chamber method (Scholander *et al.* 1965). In all cases, two branches were taken from the same individual shrub with one branch serving as a treatment (freeze–thaw) and the other as a control (non-freeze–thaw). Usually two stem segments were taken from each branch to estimate the degree of xylem embolism.

After various treatments involving either artificial dehydration, rapid freeze–thaw events or gradual freeze–thaw events (see below), entire branches were submerged in water and alternately trimmed at each end to finally produce a stem segment 10 cm long and 6–8 mm in diameter. The cut ends of stem segments were finally shaved with a

new razor blade and placed in a tubing manifold accommodating 12 stem segments at a time, similar to that described by Sperry *et al.* (1988). The manifold allowed the measurement of hydraulic conductance (K_h) before and after the removal of gas emboli from xylem conduits. Briefly, a degassed solution of 10 mol m^{-3} citric acid was filtered through a 0·1 μm filter and allowed to flow through one stem segment at a time under about 3 kPa of hydrostatic pressure. This pressure was empirically determined to be too low to force gas bubbles through embolized vessels cut open at both ends (Jarbeau, Ewers & Davis 1995). The flow through each stem segment was channelled into a flask on an analytical balance and flow rate was measured as mass increase per unit time (g s^{-1}). This was converted to volume flow rates (m^3 s^{-1}) at standard temperature (25 °C) and divided by the pressure gradient across the stem segment (MPa m^{-1}) to calculate hydraulic conductance per pressure gradient (K_h) in units of m^4 MPa^{-1} s^{-1} (Jarbeau, Ewers & Davis 1995). The initial measurement of K_h was followed by two or more subsequent measurements, each after 1 h of high-pressure perfusion (175 kPa) to remove gas emboli from vessels and tracheids. The perfusion/measurement cycle was repeated until stable maximum values were achieved. The removal of emboli resulted in an increase in K_h relative to initial measurements. The difference between initial and final (maximum) values was used to calculate percentage loss in hydraulic conductivity due to gas embolism (Sperry, Donnelly & Tyree 1988).

Hydrated and dehydrated branches exposed to a rapid freeze–thaw event

In order to test the hypothesis that a prolonged summer drought might increase vulnerability to freezing-induced embolism, branches of *R. laurina* and *C. megacarpus* were either kept hydrated by placement in a water-filled bucket or artificially dehydrated to various levels in the laboratory as described above. Branches were collected from a 23-year-old mixed stand of *R. laurina* and *C. megacarpus*, between May and August of 1993, growing at our warm site in Malibu. Two branches were collected from each individual shrub for freeze–thaw treatments; one was used as a control to estimate native embolism or the influence of water stress on embolism formation; the second branch was used to determine any additional impact of a freeze–thaw event on embolism formation. The total number of branches measured for each species and treatment ranged between 45 and 55. After determination of water potentials, control and treated branches were enclosed in a moist plastic bag. Controls were left in a vertical position in an air-conditioned laboratory (22–24 °C) while treated branches were placed vertically in an upright freezer. Treated branches were left in the freezer overnight, undergoing a cooling rate of about 0·8 °C min^{-1} from room temperature down to –20 °C. Treated branches were then removed from the freezer, kept in a vertical position, and allowed to thaw at a rate of 0·8 °C min^{-1} until they returned to room temperature. Cooling and warming rates of stem segments were determined by spring-loading one thermocouple, held in place with masking tape, on each of six stems and recording temperatures every 10 s, averaged over 1 min intervals, with a datalogger (Model 21 X, Campbell Scientific, Inc. Logan, UT). This simple method of rapid freeze–thawing of branch tissues approximately follows that described by Sperry & Sullivan (1992).

Hydrated branches exposed to a gradual freeze–thaw event

A gradual freeze–thaw technique was also employed to determine whether the rate of warming or cooling had a significant effect on the amount of embolism formation in stems. We used the micrometeorological data collected on *Rhus* plants at our Cold Creek Canyon site (described above). The natural rates of cooling and warming were calculated from these data and duplicated in the laboratory with a freezing apparatus simulating a gradual freeze–thaw event (Fig. 1b). The rate of cooling was set at 0·08 °C min^{-1} when decreasing from 15 to 0 °C and at 0·02 °C min^{-1} when decreasing from 0 to –20 °C. The warming rate was 0·08 °C min^{-1} from –20 to 15 °C. A computer program to control cooling and thaw rates was downloaded to the datalogger which controlled a refrigerated circulating bath (Model ULT-80DD, Neslab, Inc., Portsmouth, NH) that pumped methanol through 60 m of coiled, copper tubing inside a 0·2 m^3 aluminium can (Fig. 1b). The aluminium can was inverted on a stand and insulated with 120-mm-thick foam rubber at the open end and five layers of aluminium airseal insulation (Reflectix Inc., Markeiville, IN) on the sides and bottom. The inside of the can was painted black to increase radiative heat exchange between the wall of the chamber and the plant material. No fans were used because mechanical vibrations were found to induce ice nucleation prematurely in stem tissues at artificially high temperatures. The maximum air temperature gradient along the length of the can was found to be less than 1 °C at temperatures down to –20 °C.

Hydrated branches were kept in a tall, water-filled bucket. The bucket had a central dowel pole attached to its bottom and a perforated lid at its top to provide branch support. At least two stem segments were selected on each of the three branches placed in the cooling chamber. Thermocouples were attached to stem segments of the branch by spring-loading the wires and securing lead wires with masking tape. Two thermocouples monitored air temperature near the topmost and bottom-most leaves in the cooling chamber. Maximum differences in these two air temperatures did not exceed 1 °C. Control branches were held vertically in a bucket of water and covered by a dark, moist plastic bag. The freeze–thaw treatment ran overnight and the hydraulic conductivity of stem segments from the control and treated groups was measured the following day (see above). We collected paired branches from 18 individuals of each species (n = 18) at the warm site in Malibu between May and August of 1994 (for the

hydrated experiments) and between May and July of 1995 (for the dehydrated experiments, using *C. megacarpus*), following the procedure given above.

Variation of thaw rate

We also varied thaw rates to determine whether they would influence the degree of xylem embolism formation. Experiments were performed mostly on C. *megacarpus* because *R. laurina* became very highly embolized even when well hydrated and when given the most gradual thaw rate. The technique was the same as that described above, except that the thaw rate for different experiments was increased from the natural thaw rate of 0·08 °C min^{-1}, measured in the field, to artificially elevated thaw rates of 0·33, 0·66, 1·25 and 2·5 °C min^{-1}. Branches were collected between May and August 1994 at the warm site in Malibu. The sample size ranged between 9 and 18 for each thaw rate; the pooled sample size for the unfrozen controls was 32.

Variation in freezing intensity

Additional experiments were carried out on hydrated plants of *Ceanothus megacarpus* to determine whether freezing intensity (minimum freezing temperature) influenced the degree of xylem embolism formation. Natural freeze–thaw rates were used (thaw rate 0·08 °C min^{-1}) while the minimum temperature was lowered from –5 °C down to –20 °C. Branches were collected between May and August 1994 at the warm site in Malibu (n = 18 for each treatment).

Statistical analysis

All values for percentage loss in hydraulic conductivity due to gas embolism were arcsin transformed before statistical comparisons were made. Unfrozen control and freeze–thaw treated groups were compared using an unpaired Student's t-test and considered to be significantly different at $P < 0{\cdot}05$. Comparisons among more than two treatments were performed by one-way ANOVA followed by Fisher's Protected Least Significant Difference Test at $P < 0{\cdot}05$. A two-tailed Dunnett post hoc test at $P < 0{\cdot}05$ was used to compare combined control values against the different thaw rates.

RESULTS

Natural freeze–thaw events at the cold site

Leaf, stem and air temperatures at the cold study site depended upon the microenvironment. Leaf temperatures on the coldest night of 1992 are shown in Fig. 3. The coldest nights were typically clear and followed by relatively warm, sunny days, with a difference between minimum and maximum for a particular day being 25 °C or more. The upper, more exposed leaves (top in Fig. 3) became colder than the lower leaves by as much as 1·4 °C, and

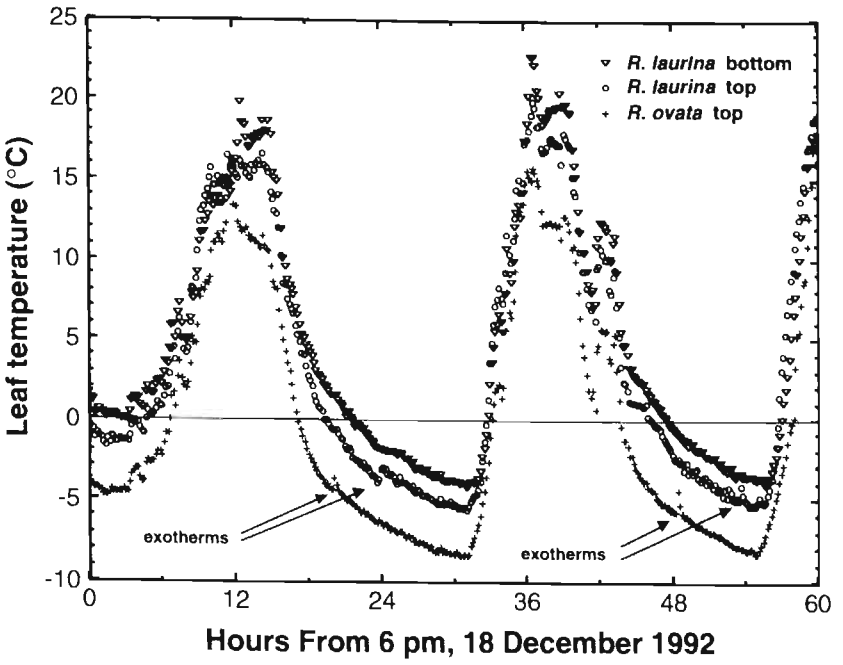

Figure 3. Leaf temperatures on the hill (*Rhus laurina*, top leaves and bottom leaves) and valley (*Rhus ovata*, top leaves) at Cold Creek Canyon (see Figs 2A & B) during a radiation freeze on the nights of 19–20 December 1992. The topmost leaves of *R. laurina* sustained severe damage (Table 1) followed by 95% embolism of stems (Fig. 4). Because *R. ovata* was in the valley, its topmost leaves were 3·1–3·8 °C lower in temperature than the topmost leaves of *R. laurina* on the hill. Note that exotherms of topmost leaves of both *R. laurina* and *R. ovata* were around – 5 °C, suggesting that freezing of leaf tissue occurred. This was not the case for the bottom leaves of *R. laurina* on the hill. Since temperatures were measured every 60 s and then the average for each 10 min interval was calculated and stored in datalogger memory, exotherm events are not discrete recordings. We also measured temperatures of the air and stems adjacent to top and bottom leaves of both species, but the data are not shown for simplicity. Air temperatures usually ran about 0·3 °C higher than leaf temperatures at night and stem temperatures were usually less than 0·1°C higher than leaf temperatures.

colder than the air temperature by as much as 0·4 °C (data not shown). Stem temperatures were not significantly different from leaf temperatures ($P > 0{\cdot}3$ to $P > 0{\cdot}9$) and mean values taken over 10 min intervals throughout the night were always within 0·2 °C of each other (data not shown). At the cold site, temperatures of the topmost leaves of *R. laurina* near the ridge crest were 3·1–3·8 °C higher than those of topmost leaves of *R. ovata* at the valley bottom (Figs 2A & 3). *Rhus laurina* was restricted to the hilltop, whereas *R. ovata* occurred at both hilltop and valley microsites (Fig. 2A). On the nights of 19 and 20 December 1992, the leaves and stems of *R. ovata* froze, as evidenced by an exotherm (Fig. 3), whereas only the upper leaves and upper stems of *R. laurina* froze on those nights (Fig. 3). Leaf vitality, estimated by photosynthetic fluorescence, vital stain and leaf coloration (Fig. 2D), showed that the upper leaves of *R. laurina*, which froze, died (Table 1), and that the stems of this species became 95% embolized (Fig. 4). In contrast, the bottom-most leaves of *R. laurina* and all leaves of R. *ovata* remained alive (Table 1) and the stems of *R. ovata* were only 79% embolized (Fig. 4). By January 1993, the upper stems of *R. laurina* had died back

	F_vF_m				
	Hours before the freeze		Hours after the freeze		Post-freeze Mesophyll cells alive (%)
	58 h	10 h	10 h	34 h	
R. laurina (a) (top leaves)	0·722 (0·049)	0·640 (0·045)	0·091 (0·061)	0·179 (0·026)	1·4 (1·4)
R. laurina (b) (bottom leaves)	0·802 (0·015)	0·761 (0·024)	0·717[a] (0·025)	—	98·2[a] (1·5)
R. ovata (c) (top leaves)	0·759 (0·014)	0·681 (0·016)	0·677[a] (0·012)	—	99·9[a,b] (0·1)
R. ovata (d) (bottom leaves)	—	0·764[a] (0·012)	0·656[a] (0·010)	—	100·0[ab] (0·0)
P	>0·24	<0·02	<0·0001	—	<0·0001

Table 1. Comparison of variable leaf fluorescence (F_v/F_m) before and after a freezing event (Fig. 3) on 19 December 1992, at Cold Creek Canyon. Also shown is the viability of leaf mesophyll cells (mesophyll cells alive) after the freezing event. Measurements were made in the upper canopy (top leaves) and understory (bottom leaves) of co-occurring *Rhus laurina* and *Rhus ovata*. Mean values were compared by one-way ANOVA followed by a Fisher's Protected Least Significance Test. Letters in superscript indicate significant difference from (a) *R. laurina* top leaves, (b) *R. laurina* bottom leaves, (c) *R. ovata* top leaves or (d) *R. ovata* bottom leaves at $P < 0·05$, $n = 5$

while the lower stems and leaves remained viable (Fig. 2B). During this same time period at the warm study site in Malibu, where freezing temperatures did not occur, native embolism values were only 50% for *R. laurina* and 34% for *R. ovata* (Fig. 4).

Hydrated and dehydrated branches exposed to a rapid freeze–thaw event

Ceanothus megacarpus was more resistant to freezing-induced as well as to water stress-induced embolism than *R. laurina* (Figs 5a & b). In both species, freezing increased the amount of embolism, unless the water stress by itself was already sufficient to cause 100% embolism. *R. laurina* became 50% embolized due to water stress at a xylem tension of –2·4 MPa and about 98% embolized by a freeze–thaw cycle at all hydration levels. In contrast, *C. megacarpus* became 50% embolized at a water stress of –8·6 MPa and 98% embolized or greater due to freezing only after substantial dehydration (–3 MPa and lower).

Hydrated branches exposed to a gradual freeze–thaw event

Compared to a rapid freeze–thaw cycle, gradual freezing (0·02 °C min^{-1}) and thawing (0·08 °C min^{-1}), which simulated a natural freeze–thaw event (Fig. 3), resulted in significantly less embolism for *C. megacarpus*, but not for *R. laurina* (Figs 5a & b). Embolism formation in hydrated *C. megacarpus* was 35% during a gradual freeze–thaw event versus a significantly higher 74% for a rapid freeze–thaw event (d.f. = 17, $t = 5·3$, $P < 0·0001$). Similarly, at –5 MPa, embolism was 90% during a gradual freeze–thaw event versus a significantly higher 100% for a rapid freeze–thaw event (d.f. = 22, $t = 4·2$, $P < 0·001$). When the xylem water potential was –8·5 MPa a gradual freeze–thaw event resulted in an embolism level of 95%.

In hydrated *R. laurina*, the mean percentage loss in K_h was reduced from 98% during a rapid freeze–thaw event to 91%

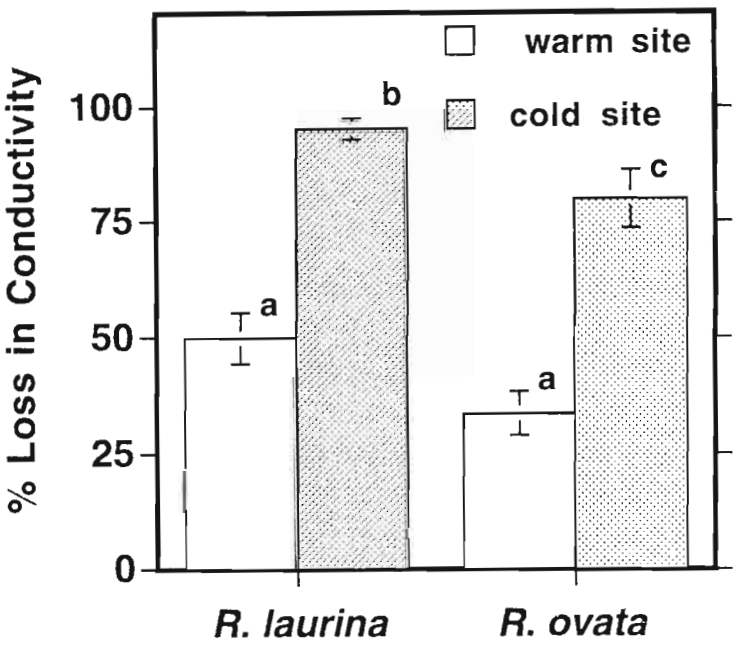

Figure 4. Comparison of percentage loss in hydraulic conductivity due to gas embolism in stem xylem of *Rhus laurina* and *Rhus ovata* at a warm site (coastal exposure of the Santa Monica Mountains at Malibu) and a cold site (Cold Creek Canyon, 4 km inland from Malibu) in early January 1993. Nighttime temperatures in winter rarely reach 0 °C at the warm site but frequently reach –8 °C (e.g. 19–20 December 1992; see Fig. 3) and sometimes –12 °C at the cold site (Boorse 1995). A one-way ANOVA (d.f. = 44, $F = 34·6$, $P < 0·0001$) followed by a Fisher's Protected Least Significant Difference Test indicated that embolism levels were not significantly different ($P > 0·09$) at the warm site but were at the cold site ($P < 0·001$). Error bars represent ± 1 SE, $n = 12$. Letters in common on bar graphs indicate no significant difference.

during a gradual freeze thaw event. However, these values were not significantly different (d.f. = 29, $t = 1·718$, $P > 0·09$).

Variation of thaw rate

An increase in thaw rate above 0·66 °C min^{-1} significantly increased percentage loss in K_h for *C. megacarpus* (Fig. 6). A one-way ANOVA indicated that there was no significant difference in embolism formation among thaw rates of

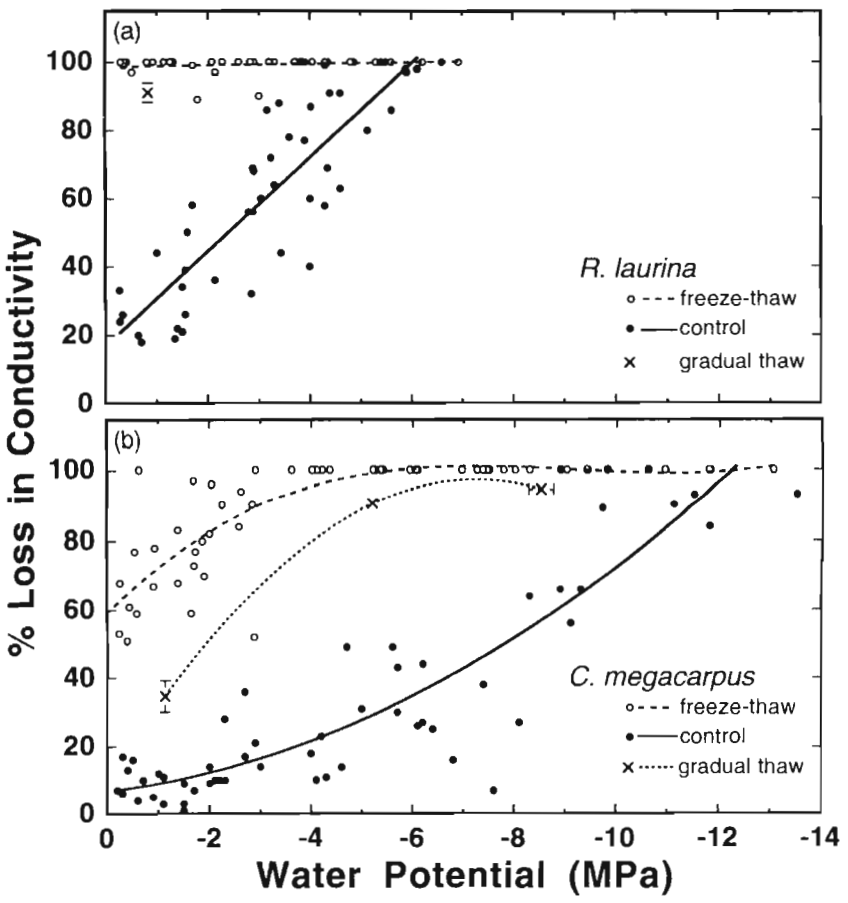

Figure 5. Comparison of percentage loss in hydraulic conductivity due to gas embolism in stem xylem after water stress and/or freeze–thaw treatments in (a) *Rhus laurina* and (b) *Ceanothus megacarpus*. Individuals of both species underwent a rapid freeze and a rapid thaw (open circles) in a –20 °C freezer. The data were fitted with a linear equation for both water stress ($y = -13{\cdot}81x + 16{\cdot}91$, $r^2 = 0{\cdot}75$) and rapid freeze–thaw ($y = -0{\cdot}234x + 98{\cdot}56$, $r^2 = 0{\cdot}04$) for *R. laurina*, but by polynomial equations for water stress ($y = 0{\cdot}073x^3 + 1{\cdot}84x^3 + 4{\cdot}87x + 13{\cdot}04$, $r^2 = 0{\cdot}81$) and rapid freeze–thaw ($y = -0{\cdot}063x^3 - 1{\cdot}719x^2 + 14{\cdot}94x + 59{\cdot}08$, $r^2 = 0{\cdot}66$) for *C. megacarpus*. Another group of individuals from both species (x symbols, $n = 18$ for each species) were subjected to a gradual cool and 0·08 °C min^{-1} thaw simulating a normal freeze–thaw event measured under natural field conditions (Fig. 3). Since embolism levels were significantly reduced for gradual freeze–thaw rates of hydrated C. *megacarpus*, additional measurements were made at dehydration levels of –5·2 and –8·5 MPa and the three points fitted with a polynomial equation ($y = -1{\cdot}69x^2 - 24{\cdot}35x + 9{\cdot}335$, $r^2 = 1{\cdot}00$). Error bars on symbols represent ± 1 SE, $n = 18$. Error bars are not shown when eclipsed by symbols.

0·08, 0·33, and 0·66 °C min^{-1}. There were significant differences in percentage loss in K_h when the thaw rate was higher than 0·66 °C min^{-1} (d.f. = 5, $F = 39{\cdot}1$, $P < 0{\cdot}0001$). There was no significant difference between the controls using a one-way ANOVA, so the values for controls were pooled ($n = 32$). A two-tailed Dunnett test showed significant differences between the control and thaw rates of 0·33, 0·66, 1·25 and 2·5 °C min^{-1} (asterisks in Fig. 6).

Variation in freezing intensity

Raising the minimum freezing temperature in hydrated *C. megacarpus* from –20 to –5 °C did not cause a significant decrease in percentage loss in K_h (Fig. 7) (d.f. = 34, $t = 0{\cdot}49$, $P > 0.63$). At both temperatures there was a significant difference between the unfrozen control branches and branches exposed to a freeze–thaw event (–5 °C: d.f. = 34, $t = -2{\cdot}47$, $P < 0{\cdot}019$) (–20 °C: d.f. = 22, $t = -5{\cdot}44$, $P < 0{\cdot}0001$). A single freeze–thaw event increased percentage loss in K_h (increased embolism) by 13% for the –5 °C treatment and 17% for the –20 °C treatment (Fig. 7).

DISCUSSION

The chaparral plant community of southern California must be resistant to multiple stress factors, including protracted summer drought, periodic wildfires, and, in some regions, freezing air temperatures. Drought, especially when combined with freezing, could block water transport in woody stems, leading to shoot dieback. Available data suggest that reversal of embolism does not occur in mature chaparral shrubs (Kolb & Davis 1994; Williams, Davis & Portwood 1997). In the case of non-sprouting species, such as *C. megacarpus*, shoot dieback would probably result in whole-plant death. Among sprouting species, however, such as *R. laurina*, shoot dieback would only represent loss in construction costs of accumulated shoot growth. As long as shoot dieback is not too frequent, there is nearly 100% sprouting success after shoot removal by disturbance (Zedler, Gautier & McMaster 1983; Frazer & Davis 1988; Thomas & Davis 1989). However, near the cold edge of the range of *R. laurina,* frequent low temperature events, for example several years in a row, could eventually lead to whole-plant death (Misquez 1990).

With such distinct differences in life history characteristics between these two species of chaparral shrubs, it is not

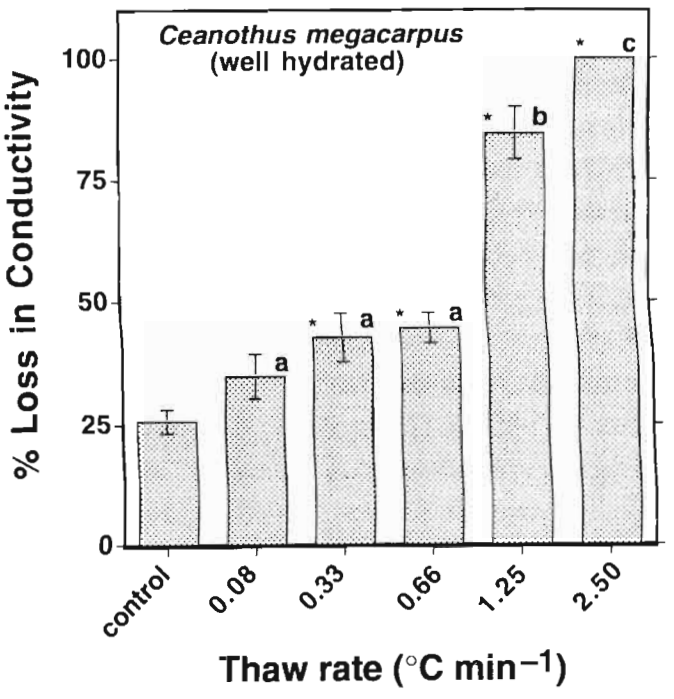

Figure 6. Relationship between the thaw rate of well-hydrated *Ceanothus megacarpus* and percentage loss in hydraulic conductivity due to gas embolism formation in stem xylem. The asterisks represent a significant difference from the pooled control ($n = 32$) by a two-tailed Dunnett test at $P < 0{\cdot}05$. Error bars represent ± 1 SE ($n = 18$, 0·08 °C min^{-1}; $n = 9$, 0·33 °C min^{-1}; $n = 9$, 0·66 °C min^{-1}; $n = 18$, 1·25 °C min^{-1}; $n = 9$, 2·5 °C min^{-1}). Letters next to error bars represent significant difference by one-way ANOVA followed by a Fisher's Protected Least Significant Difference Test at $P < 0{\cdot}05$.

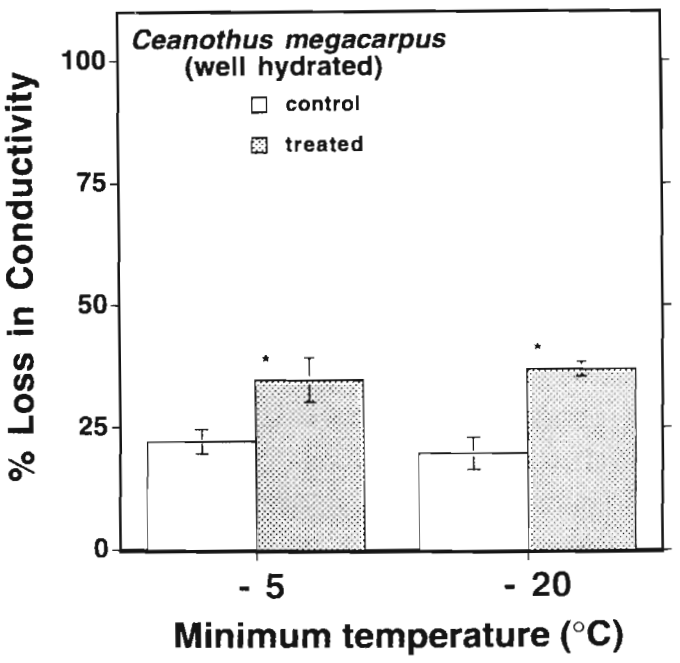

Figure 7. Relationship between freezing intensity (minimum temperature) and percentage loss in hydraulic conductivity due to gas embolism formation in stem xylem of well-hydrated *Ceanothus megacarpus*. Freeze–thaw rates were gradual (thaw rate of 0·08 °C min^{-1}), similar to those observed under field conditions (Fig. 3). Error bars represent ± 1 SE. The asterisks represent significant difference by unpaired Student's *t*-test at $P < 0·05$ between unfrozen controls ($n = 18$, –5 °C; $n = 6$, –20 °C) and freeze–thaw treated plants ($n = 18$, –5 °C; $n = 18$, –20 °C).

surprising that in the face of the same environmental stresses of drought and winter freezing, co-occurring *C. megacarpus* and *R. laurina* have markedly different sensitivities to water stress, freezing and their combination. The differential vulnerability to water stress-induced embolism observed between *R. laurina* and *C. megacarpus* in this study is consistent with previous studies. Resprouts of *Rhus laurina* were reported to become 50% embolized at –1·6 MPa (Jarbeau, Ewers & Davis 1995) while *C. megacarpus* became 50% embolized at –11 MPa (Kolb & Davis 1994). In the present study, *R. laurina* became 50% embolized due to water stress at a tension of –2·4 MPa and *C. megacarpus* became 50% embolized at a water stress of –8·6 MPa. This pattern corresponds to differential rooting depths (*R. laurina*, deep roots; *C. megacarpus*, shallow roots), minimum seasonal water potential of stem xylem (*R. laurina*, –2·5 MPa; *C. megacarpus*, –9 MPa), and post-fire seedling survivorship (*R. laurina*, 0·01%; *C. megacarpus*, 42%; Thomas & Davis 1989; Saruwatari & Davis 1989). Furthermore, it is consistent with differences in phenology, differences in life history characteristics and the evidence of niche differentiation between the two species (Davis, Kolb & Barton 1997).

Why, in southern California, is *R. laurina* primarily restricted to coastal regions? Freezing-induced dieback is a plausible explanation. At issue is whether the dieback is due more to freezing damage to living tissue (Boorse 1995), or to freezing-induced embolism. On both counts *R. laurina* appears to be quite vulnerable to low temperatures. With winter acclimation, the LT_{50} (temperature for 50% leaf death) appears to be about –5 °C at our warm site and about -6 °C at our cold site (Boorse 1995). Since freezing of xylem water in this species occurs at –3·6 °C (Boorse 1995), the present study shows that the stems, with freezing-induced embolism, may be more vulnerable to low temperatures than the leaves. With 98% embolism of the stem, the leaves would probably soon die from dehydration, even if they survived the actual freeze–thaw event (barring embolism reversal, which appears to be unlikely for mature chaparral shrubs; cf. Kolb & Davis 1994; Williams, Davis & Portwood 1997). Following natural freeze–thaw events, leaves of *R. laurina* have been described as desiccated (Misquez 1990).

Why, in southern California, is *C. megacarpus* restricted to the coastal exposures, with perhaps an even more restricted distribution than *R. laurina*? The length of the seasonal drought period in summer tends to be longer in the coastal areas than inland; thus the great drought tolerance of *C. megacarpus* would be of a distinct advantage along the coast (Miller & Poole 1979). In the northern limits of its distribution, *C. megacarpus* tends to be limited to the south-facing, ocean-facing slopes of the Santa Ynez and Santa Monica Mountains and, on nearby islands (Schlesinger *et al.* 1982), sites where freezing might be extremely rare. At higher elevations, above 900 m, *C. megacarpus* intergrades with and is eventually replaced by *C. crassifolius* in the Santa Ynez Mountains (Schlesinger *et al.* 1982; Nicholson 1993). Similarly, *C. megacarpus* is replaced by *C. crassifolius* in cold valleys of the Santa Monica Mountains (e.g. at our cold study site the dominant chaparral species, as shown in Fig. 2A, is *C. crassifolius*). Also, *C. spinosus* (a sprouter), which often co-occurs with *C. megacarpus*, generally extends into more northerly aspects and higher altitudes in the Santa Ynez Mountains (Nicholson 1993) and into cold valleys in the Santa Monica Mountains (personal observation). Could these patterns reflect a lack of low temperature tolerance for *C. megacarpus*? Unlike *R. laurina,* which has leaves that are quite sensitive to freezing temperatures, the LT_{50} for leaves of *C. megacarpus* are about –9 °C, which suggests that the latter are not particularly sensitive to low temperatures (Boorse 1995). Also, unlike *Rhus laurina*, which is extremely vulnerable to freezing-induced embolism at all water potentials, freezing has to be combined with severe water stress to promote high, potentially fatal embolism in *C. megacarpus*. However, such conditions do occur in the chaparral. If the first seasonal cold front arrived in late autumn, prior to significant rainfall, *C. megacarpus* would still be under very low water potential, as much as –9 MPa for adults in late December (Kolb & Davis 1994), at the usual time for the first winter freezing events. Even lower water potentials, as low as –12 MPa, have been reported for smaller, younger (6-year-old) shrubs of this species (Schlesinger *et al.* 1982). Our data indicate 95% or greater embolism caused by a *single* freeze–thaw event under such conditions (Fig. 5b), which would probably cause whole-plant death for this non-sprouting species. Thus the extreme drought tolerance of *C. megacarpus*, which appears to be greater than for any of the six species of *Ceanothus* in the Santa Monica Mountains (Davis, Kolb &

Barton 1997), allows plants with remarkably low water potentials to persist. However, when such plants are exposed to freezing temperatures, whole-plant death might result. Another *Ceanothus* species, *C. crassifolius,* a non-sprouting species which dominates at our cold study site (Fig. 2A), does not experience such low water potentials (data not shown) and perhaps for this reason does not suffer as much from freezing-induced embolism.

The higher vulnerability of *R. laurina* to freezing-induced embolism, when compared to *C. megacarpus*, is consistent with the model proposed by Ewers (1985). Although *R. laurina* and *C. megacarpus* often grow side by side, their xylem vessel diameters are very different. Using similar diameter stems as in the present study, *R. laurina* was found to have mean and maximum vessel diameters of 40 and 80 μm, respectively, whereas *C. megacarpus* had mean and maximum vessel diameters of 21 and 42 μm, respectively (data not shown). Consequently, specific conductivity was found to be much higher for *R. laurina* at 6·7 m^2 MPa^{-1} s^{-1} than for *C. megacarpus* at 1·8 m^2 MPa^{-1} s^{-1} (data not shown). The impact of freezing-induced embolism on hydraulic supply in *R. laurina* may be far greater than indicated by its 2-fold larger vessel diameter since hydraulic conductance is a function of vessel diameter to the fourth power, following the Hagen-Poiseuille relation for theoretical conductivity (Tyree, Davis & Cochard 1994). This would especially be true if preferentially larger conduits within a plant were more susceptible to embolism caused by freezing, as found for other species (Cochard & Tyree 1990; Sperry & Sullivan 1992; Sperry & Saliendra 1994; LoGullo & Salleo 1993).

In *R. laurina*, with its relatively large vessels for a chaparral shrub, there may be a trade-off between superior hydraulic efficiency and greater vulnerability to xylem dysfunction due to freezing. Furthermore, because adult plants of *R. laurina* avoid low water potentials, by virtue of an extremely deep root system combined with relatively efficient water conduction (Thomas & Davis 1989; Jarbeau, Ewers & Davis 1995), embolism induced by freezing for *Rhus laurina* appears to be more significant ecologically than embolism caused by water stress.

The rate of thaw appears to be an important factor in determining the amount of embolism formation in *C. megacarpus*. Faster thaw rates presumably allow less time for the gas bubbles in xylem conduits to go back into solution (Sperry 1995). Sperry & Sullivan (1992) found xylem water potentials required to cause 50% embolism in freeze–thaw stems to be 1–2 orders of magnitude more negative than predicted. They proposed several possible reasons for this: (1) pressures increase upon freezing and thus may force bubbles back into solution at the time of thaw (cf. Hammel 1967); (2) since xylem water is degassed by freezing there is a strong tendency for bubbles to redissolve at the time of thaw; (3) xylem water may flow in advance of ice formation and could refill some of the previously embolized vessels, and (4) if no transpiration occurs, the pressure will not decrease to prefreezing values until thawing is complete. For items (1), (2) and (4), the time for bubbles to dissolve, i.e. the rate of thaw, may be important. Sperry & Sullivan conclude that embolism formation may depend both on rates of freezing and thawing and on the direction of ice propagation in stems. In our case, branches stood vertically in a bucket of water and freezing was at the distal, canopy end, similar to freezing by radiation in the field at night.

Our results indicate that rapid freeze and thaw rates during artificial experiments in the laboratory (Sperry & Sullivan 1992) may cause artifactual formation of embolism. Such experiments may not quantitatively represent the amount of embolism that is formed during winter freezing in nature. In the chaparral at least, low temperature episodes usually result in gradual freeze–thaw events.

Although not examined in our study, the number of freeze–thaw cycles may also influence the degree of embolism formation in temperate climates. When a cold spell occurs in the Mediterranean-type climate of California, chaparral shrubs usually encounter sub-zero temperatures at night, thaw at dawn just prior to the onset of transpiration, and freeze again if subzero temperatures are reached the next night (cf. Fig. 3). Evergreen plants with large volume conduits would be at an extreme disadvantage with multiple freeze–thaw events because the amount of embolism could be additive for each freeze–thaw cycle (Sperry & Sullivan 1992; Sperry 1995).

Studies performed by Sperry *et al.* (1994) comparing embolism in woody plants in Utah and Alaska indicate that there is not an increase in the amount of embolism due to intensity (minimum temperature) of the freeze. In contrast, LoGullo & Salleo (1993) found minimum temperature and leaf death to affect embolism in *Quercus ilex.* Also, Pockman & Sperry (1996) found embolism to increase linearly as minimum temperature decreased from – 11 to –20 °C in the evergreen desert shrub *Larrea tridentata.* Our results comparing embolism formation in *C. megacarpus* at –5 and –20 °C are consistent with the findings of Sperry *et al.* (1994). However, we did not vary the minimum temperature in *R. laurina,* where the minimum temperature for the embolism studies was always kept at –20 °C.

Conclusion

We conclude that, in studies on freezing-induced embolism among chaparral shrubs, it is important to consider the hydration of the plant, the minimum temperature and the rates of cooling and warming, each of which can affect embolism levels. Our study shows that both water stress- and freezing-induced embolism need to be included as possible factors limiting the growth, survival and distribution of chaparral species. For *R. laurina,* freezing-induced embolism appears to be a threat to shoot survival regardless of the plant's hydration level. In

contrast, for *C. megacarpus*, severe freezing-induced embolism results only when the plants are already under extreme water stress.

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